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Accumulation, Whole-Body Depletion, and Debromination of Decabromodiphenyl Ether in Male Sprague−Dawley Rats Following Dietary Exposure

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Decabromodiphenyl ether (BDE-209) is the major component in the flame-retardant formulation DecaBDE which is incorporated into numerous consumer goods ranging from upholsteries to electronics. Because of the high volume of DecaBDE produced, its presence in consumer products and the environment, and the finding of BDE-209 in the blood of exposed workers, the extent of bioavailability, persistence, and potential debromination are important issues. To measure the bioconcentration, distribution, reductive debromination, and whole-body half-lives of BDE-209 after multiple low doses in an animal model, we dosed rats with a commercial DecaBDE (0.3 μg/g of diet) for 21 days and measured tissue polybrominated diphenyl ether levels during a 21 day withdrawal period. BDE-209, three nona-BDEs, and four octa-BDEs accumulated in the rats and distributed proportionately throughout the body. Only 5% of the total BDE-209 dose was present as parent compound in the rats after 21 days of dosing and <4% in the feces, suggesting extensive metabolism. A nona-BDE (BDE-207) and two octa-BDEs (BDEs-201 and -197) appeared to form via *meta*-debromination(s) of BDE-209 to a minimal extent (1% of the total BDE-209 dose). The whole-body half-lives tended to increase with decreasing bromination; however, two octa-BDEs, presumably forming from debromination, increased in the rats after 21 days of withdrawal and demonstrated the potential for BDE-209 to form more persistent lipophilic compounds in vivo.

Introduction

Polybrominated diphenyl ethers (PBDEs) are a class of additive flame retardants used in a variety of consumer products including textiles, electronics, and appliances. Three major formulations of PBDEs have been produced and marketed with varying degrees of bromination: PentaBDE, OctaBDE, and DecaBDE. DecaBDE has the highest rate of production of the three formulations with a market demand of 56100 metric tons in 2001, compared to 3790 tons for OctaBDE and 7500 tons for PentaBDE (*1*). The PentaBDE and OctaBDE formulations are composed mainly of the tetra- to octabrominated diphenyl ethers, and both have recently been restricted from production and use in the EU due to concerns of environment persistence, bioaccumulation, and adverse health effects (*2*, *3*). These two formulations were also voluntarily phased out of production in the U.S. at the end of 2004 (*1*). The DecaBDE formulation remains in production but is undergoing further review with focuses on toxicokinetics, degradation to more persistent compounds, and neurotoxic effects (*4*).

The DecaBDE formulation is composed of mainly BDE-209, the perbrominated congener. Studies have shown that BDE-209 is absorbed by rats, fish, and seals from the diet (*5*−*8*). Studies quantifying the bioavailability of BDE-209 have produced a range of results. In four studies with rats, the amount of BDE-209 bioavailable from the diet ranged from not detectable after 1 year of feeding to 26% as calculated from pharmacokinetic modeling (*5*, *6*, *9*, *10*). These differences in bioavailability may be attributed to a number of factors including high detection limits in the earliest study, matrix effects, or dose size. In some studies the dose was dissolved in a lipophilic, oily matrix which may have provided better absorption than a dry dose mixed in the feed. The bioavailability of BDE-209 also appeared to be dose dependent in one study, ranging from 0.2% of a high dose (~75 mg/rat) to 1.1% of a lower dose (~400 μg/rat), perhaps due to limitations in intestinal uptake or increased metabolism from the higher dose (*5*).

Although bioaccumulation appeared low for BDE-209, metabolism was extensive with up to 70% of a single dose biotransformed in rats (*6*), suggesting far greater bioavailability than the earlier reported 1.1% (*5*). In fish BDE-209 is debrominated to lower brominated diphenyl ethers including penta- to nona-BDEs, which may have greater persistence than the parent compound (*11*, *12*). Recently it was suggested that dairy cattle also reductively debrominate highly brominated diphenyl ethers (*13*). In rats BDE-209 was mainly metabolized to bound residues or hydroxymethoxy- or hydroxy-substituted diphenyl ethers having five to nine bromines (*6*, *10*). Trace amounts of three nona-BDEs were identified in one study (*6*), indicating the occurrence of reductive debromination, but it was not confirmed whether debromination was due to metabolism or an artifact of degradation during the cleanup process. In humans debromination of BDE-209 has been hypothesized to account for the presence of octa- and nona-BDEs in the serum of occupationally exposed workers (*14*) and to explain the increasing proportion of BDE-153 in human milk samples measured in recent years (*15*). However, except in fish, reductive debromination has not been confirmed in other animal systems.

Although BDE-209 is rapidly metabolized, it has been found to accumulate in the livers of rats (*6*), in the livers and muscle of trout (*12*), and in the adipose tissue of gray seals (*8*), showing potential for persistence. The serum half-life of BDE-209 ranged from 2 to 15 days in rats (*10*), gray seals (*8*), and occupationally exposed workers (*16*). Corresponding depletion rates of BDE-209 from other body compartments have not been measured, although it was estimated that 11−15% of a dose remained in the adipose tissue of gray seals after 1 month of withdrawal (*8*). The object of this study was to measure the bioconcentration, distribution, debromination, and whole-body half-lives of BDE-209 after multiple low doses using rats as a model system.

Experimental Section

Twenty-six male Sprague−Dawley rats (Harlan, Indianapolis, IN) were raised in-house from 21 to 65 days of age with free access to feed (Rat Diet, PMI Nutrition International, LLC, Brentwood, MO; 22% crude protein, 4% fat, and 5% fiber) and water. The rats were then placed in individual cages

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designed for separately collecting feces and urine and trained to eat ground rat chow (12 g) topped with corn oil (200 μL) over a 2 h period each morning. Water was available on an ad libitum basis, but feeding was restricted to a daily 2 h period. After 2 weeks of training, all rats were consuming the entire portion of feed and oil. Rats were weighed weekly to ensure that weights were maintained during the experiment.

The dose was prepared by dissolving a toluene solution (550 μL) containing 2.06 mg of DecaBDE (98.5% pure, DE-83R, Great Lakes Chemical) in corn oil (109 mL). The solution was stirred overnight under a nitrogen stream to remove most of the toluene as determined gravimetrically. The final concentration was 18.9 μg of BDE-209/mL of oil for a daily dose of 3.8 μg in 200 μL of oil/rat (0.3 μg/g of the total diet). The rats were randomly divided into dosed and control groups. Control rats ($n = 8$, 210.0 $\pm$ 7.5 g) received feed (12 g) and oil (200 μL) once a day for the entire experiment; dosed rats ($n = 18$, 200.2 $\pm$ 10.7 g) received feed (12 g) and the oil dose (200 μL) for 21 consecutive days and then the same amounts of feed and corn oil during a withdrawal period that lasted up to 21 days.

The rats were sacrificed 24 h after the last feeding by first anesthetizing with halothane/nitrous oxide and then exsanguinating by venipuncture. Dosed rats were sacrificed in groups of three on withdrawal days 0, 3, 7, 10, 14, and 21. Withdrawal day 0 was defined as 24 h following the last BDE-209 feeding. Three control rats were also sacrificed on day 0, and one control rat each was sacrificed at days 3, 7, 10, 14, and 21. Between withdrawal day 10 and day 14, the dosed and control rats maintained or slightly lost weight, so the four remaining rats were given an extra feed supplement (4 (g/day)/rat) for the last week of the experiment to improve weight maintenance. The blood (plasma), liver, gastrointestinal (GI) tract, and remaining carcass were collected from each rat. The GI tract (stomach, large and small intestines, and cecum) was rinsed with water before processing. The stomach was empty; the intestinal tract rinses were combined with feces. Feces and urine from each individual rat were collected separately each day and pooled for the entire experimental period. All samples were frozen at -20 °C immediately after collection.

The livers, GI tracts, carcasses, feces, and feed were homogenized, and subsamples were purified by a modification of a previously described procedure (*17*). Briefly, subsamples (1−10 g) were spiked with seven ^{13}C-labeled PBDE recovery standards (BDEs-28, -47, -99, -153, -154, -183, and -209) (Wellington Laboratories, Guelph, ON, or Cambridge Isotope Laboratories, Andover, MA) and extracted in an accelerated solvent extractor (Dionex Corp., Sunnyvale, CA) with 30:35:35 hexane/methylene chloride/2-propanol at 125 °C and 1500 psi. The extracts were sequentially washed with 20% aqueous potassium hydroxide, water, concentrated sulfuric acid, and water and then chromatographed on a PowerPrep instrument (Fluid Management Systems, Waltham, MA) using disposable triphasic silica and basic alumina cartridges. The PBDEs were eluted from the silica cartridges onto the alumina cartridges with hexane. The alumina cartridges were then washed with 2% methylene chloride in hexane and the PBDEs eluted with 50% methylene chloride in hexane. The dosing formulation in corn oil and the control corn oil were dissolved in hexane and applied directly to the PowerPrep system. Urine (50 mL) and plasma (3−5 g) were partitioned with saturated ammonium sulfate, ethanol, and hexane, and the dried hexane layer was further purified on the PowerPrep system. All extraction and cleanup steps were performed in opaque containers, and samples were covered or refrigerated between steps to minimize exposure to light.

Forty-two mono- to deca-BDEs were analyzed by an isotope dilution GC/MS method on a 30 m DB5-MS column at a mass resolution of >5000 (*17*). Occasionally, analysis on a 15 m DB5-MS column was used to improve recoveries and confirm the quantitation of BDE-209. The method was validated by replicate analyses of spiked liver, carcass, and plasma samples and showed good accuracy and precision (both relative standard deviation and relative error <30%) for the quantitation of most BDEs including BDEs-203, -206, and -209. Recoveries of the ^{13}C-labeled standards averaged 71−104%, and ^{13}C-BDE-209 recoveries ranged from 38% to 142%. PBDEs for which commercial standards were not available at the start of the study (BDEs-196, -197, -201, -207, and -208) were quantitated using the relative responses of BDEs-203 and -206 for octa- and nona-BDEs, respectively. Once other standards were obtained, quantitation was confirmed on a limited number of samples, and congeners were positively identified by elution times on two different GC columns (DB5MS and DB1MS) showing separation from possible coeluters.

The limits of detection (LODs) for BDE-209 and other congeners were established from laboratory blanks as 3 times the standard deviation of the mean ($n = 17$) or, for congeners not detected in the blanks, signal/noise = 3 calculated by the instrument operating system. Typical LODs per injection were 34 pg for BDE-47, 15 pg for BDE 99, 1−6 pg for other tri- to octa-BDEs, 87 pg for BDE-206, and 1650 pg for BDE-209. Because some PBDEs were present in laboratory blanks and the control rat tissues, accumulation and elimination of PBDEs from the dose were calculated by subtracting the average control rat levels from the average dosed rat levels. Further details of the analytical method and LODs are given in the Supporting Information.

Depletion kinetics were modeled with the control-subtracted data using GraphPad Prism (version 3.02, GraphPad Software, San Diego, CA). After visual observation of log-linear graphical representation of data sets for each congener and tissue, depletion curves were fit to either one-compartment ($C(t) = C_0e^{-kt}$) or two-compartment ($C(t) = Ae^{-\alpha t} + Be^{-\beta t}$) models (*18*), where C is the concentration of PBDE at a given time (t), k, α, and β are dissociation rate constants, and A and B are intercepts associated with rate constants α and β, respectively. Correlation coefficients (R^2) (observed vs predicted) were calculated for each modeled curve, and 95% confidence intervals (CIs) were calculated for half-lives derived from the one-compartment models.

Results and Discussion

The DecaBDE dose was administered at 0.3 μg/g of diet, a dose that we estimated would provide levels at least 5 times higher than our detection limits in the target tissues. The daily dose was roughly 100−1000 times lower than the daily doses used in previous studies (*5*, *6*); therefore, no acute adverse effects were expected, and none were observed. The restricted diet ensured that the dose was completely consumed by the rats and also minimized the impact of body mass change during the uptake and withdrawal periods. A 21 day withdrawal period was thought to be suitable (>8 times the half-life) on the basis of an estimated half-life for BDE-209 in rats (2.5 days) (*10*).

The DecaBDE formulation was analyzed and found to be highly pure (98.5% BDE-209) and so was used as is. Other congeners detected in the formulation included nona-BDEs, octa-BDEs, and a trace of BDE-183. The feed and corn oil had no hepta- to deca-BDEs above our detection limits and, at most, contributed less than 1% to the BDE-209 intake. Although commercial standards were not available at the start of the study for all the octa- and nona-BDEs, several were obtained later to verify identifications (Supporting Information). Due to the lack of standards, quantitation of some congeners was not exact but remained consistent throughout the study.

TABLE 1. Concentrations of PBDEs in Dosed Rat Tissues and Plasma (ng/g of wet weight ± SD) from a 21 Day Feeding Study with a DecaBDE Formulation and in Control Carcass Spiked with the DecaBDE Formulation[a]

BDE no.[b]	liver ($n = 3$)	GI tract ($n = 3$)	plasma ($n = 3$)	carcass ($n = 3$)	carcass spike ($n = 3$)	BCF
209	48.2 ± 8.9	35.9 ± 8.5	3.6 ± 0.9	14.0 ± 4.0	15.7 ± 0.8	0.05
206	0.9 ± 0.8	0.7 ± 0.3	0.04 ± 0.02	0.4 ± 0.1	0.5 ± 0.03	0.09
207[c]	7.6 ± 4.0	5.8 ± 1.6	0.5 ± 0.1	2.9 ± 0.1	0.3 ± 0.01	1.6
208[c]	1.0 ± 0.7	0.7 ± 0.2	0.05 ± 0.01	0.3 ± 0.1	0.1 ± 0.01	0.9
196[c]	0.2 ± 0.07	0.2 ± 0.06	0.004 ± 0.003	0.1 ± 0.02	0.008 ± 0.001	1.1
203	0.03 ± 0.01	0.02 ± 0.01	0.002 ± 0.001	0.02 ± 0.003	0.005 ± 0.001	0.4
197[c]	0.8 ± 0.2	0.8 ± 0.3	0.02 ± 0.007	0.4 ± 0.01	0.008 ± 0.002	12.1
201[c]	0.2 ± 0.07	0.1 ± 0.07	0.004 ± 0.002	0.08 ± 0.01	nd (0.01)	8.8
183	0.02 ± 0.01	0.04 ± 0.02	0.002 ± 0.002	0.03 ± 0.006	0.016 ± 0.002	1.6
weight (g)	6.67 ± 0.38	5.27 ± 0.59	3.18 ± 0.35	216.7 ± 6.4		
lipid content (%)	4.13 ± 0.11	6.13 ± 2.89	NA	4.32 ± 1.30		

[a] Carcass BCFs were calculated as the concentration in the carcass/concentration in the feed on a wet weight basis. Average total weights (g) and lipid contents (%) of each tissue are given. Concentrations have been corrected for the levels in the control rats. For nondetected congeners (nd), the maximum amount possible based on the detection limit is given in parentheses. NA = not assayed. [b] PBDEs are listed in reverse elution order and numbered according to the IUPAC numbering system and have the following bromine substitutions: 209 = deca; 208 = 2,2′,3,3′,4,5,5′,6,6′-nona; 207 = 2,2′,3,3′,4,4′,5,6,6′-nona; 206 = 2,2′,3,3′,4,4′,5,5′,6-nona; 201 = 2,2′,3,3′,4,5′,6,6′-octa; 197 = 2,2′,3,3′,4,4′,6,6′-octa; 203 = 2,2′,3,4,4′,5,5′,6-octa; 196 = 2,2′,3,3′,4,4′,5′,6-octa; 183 = 2,2′,3,4,4′,5′,6-hepta. [c] These values are estimates because exact standards were not available to validate the analytical method; standards were obtained later to identify congeners by GC retention time comparisons.

TABLE 2. PBDE Amounts (ng ± SD) in the Dose and in the Dosed Rat Tissues, Plasma, and Feces from a 21 Day Feeding Study with a DecaBDE Formulation and the Average Percent of Each Congener Retained and Excreted by the Rats ($n = 3$)[a]

BDE no.[b]	dose[c] ($n = 3$)	av control-subtracted amts (ng) in dosed rat tissues and feces ($n = 3$)					% of dose	
		liver	carcass	GI tract	plasma	feces	retained	excreted
209	71770 ± 6400	320 ± 60	3020 ± 810	190 ± 30	11.6 ± 4.0	2730 ± 270	5	4
206	1060 ± 330	6 ± 5	80 ± 26	4 ± 1	0.1 ± 0.1	64 ± 13	9	6
207[d]	450 ± 170	50 ± 26	620 ± 15	30 ± 6	1.7 ± 0.4	64 ± 15	155	14
208[d]	90 ± 20	6 ± 5	70 ± 20	4 ± 1	nd (0.15)	21 ± 6	85	22
196[d]	24 ± 9.9	1 ± 0.5	20 ± 3	1 ± 0.2	0.01 ± 0.01	0.7 ± 0.6	108	3
203	12 ± 4.9	0.2 ± 0.1	4 ± 1	0.1 ± 0.04	nd (0.01)	0.5 ± 0.01	35	4
197[d]	8 ± 0.7	5 ± 1	80 ± 5	4 ± 1	0.07 ± 0.03	0.9 ± 0.8	1170	12
201[d]	nd (2.4)	1 ± 0.5	20 ± 2	0.5 ± 0.4	0.01 ± 0.01	0.6 ± 0.1	845	24
183	4 ± 0.6	0.2 ± 0.1	6 ± 1	0.2 ± 0.1	0.01 ± 0.01	nd (0.01)	150	0

[a] The total amounts in the dosed tissues, plasma, and feces are corrected for the amounts in the control rats. For nondetected congeners (nd), the maximum amount possible based on the detection limit is given in parentheses. [b] PBDEs are listed in reverse elution order and numbered according to the IUPAC numbering system and have the following bromine substitutions: 209 = deca; 208 = 2,2′,3,3′,4,5,5′,6,6′-nona; 207 = 2,2′,3,3′,4,4′,5,6,6′-nona; 206 = 2,2′,3,3′,4,4′,5,5′,6-nona; 201 = 2,2′,3,3′,4,5′,6,6′-octa; 197 = 2,2′,3,3′,4,4′,6,6′-octa; 203 = 2,2′,3,4,4′,5,5′,6-octa; 196 = 2,2′,3,3′,4,4′,5′,6-octa; 183 = 2,2′,3,4,4′,5′,6-hepta. [c] Total amount of PBDEs fed to each rat over 21 days. [d] These values are estimates because exact standards were not available to validate the analytical method; standards were obtained later to identify congeners by GC retention time comparisons.

Tissue Distribution and Bioconcentration. Table 1 shows the concentrations of PBDEs that were elevated in the tissues of dosed rats compared to the controls 24 h after the last dose. In addition to BDE-209, three nona-BDEs, four octa-BDEs, and BDE-183 were observed. The control tissues had low amounts of BDE-209 (1−2 ng/g) and nona-BDEs (0.1 ng/g) due to background exposures (e.g., feed or air-borne dust), low-level laboratory contamination, or, in the case of nona-BDEs, possible degradation of BDE-209 during cleanup. However, the tissues of dosed rats contained BDE-209 at concentrations 10−20 times over those of control rats, and the hepta-, octa-, and nona-BDEs were elevated 5−240-fold over the control levels.

The concentrations of BDE-209, the nona-BDEs, and the octa-BDEs in the liver of dosed rats were 2−3 times higher than those of the carcass both on a whole weight and a lipid weight basis. Other studies of BDE-209 in rats and rainbow trout have also shown concentrations 3−20 times higher in the liver than in other major tissue compartments after oral dosing (*5*−*7*, *12*). These results are not surprising given that the liver is an organ highly perfused by the blood supply. In a 21 day feeding study in rats with the penta-BDE formulation, the lower brominated congeners (tetra to hexa) appeared to concentrate to an equal or lesser extent in the liver compared to the carcass (*19*). The disposition of these higher brominated congeners (octa to deca), therefore, appears to be different from that of lower brominated PBDEs (tetra to hexa).

Bioconcentration factors (BCFs) were calculated for each of the hepta- to deca-BDEs on the basis of the relative concentrations in carcasses and the dosed feed (wet weight basis, Table 1). Almost identical BCFs were obtained on a lipid weight basis because the carcass and feed had similar lipid percentages (data not shown). It was not established whether the PBDEs in the tissues had reached steady-state concentrations with respect to the dose; therefore, BCFs can only be considered estimates. BDEs-209 and -206 did not show high propensities to bioconcentrate (BCF = 0.5 and 0.09, respectively). However, BDEs-201 and -197 concentrated to almost 10-fold higher levels in the carcass than in the feed.

Table 2 shows total recoveries of BDE-209 and lower brominated congeners from rats fed DecaBDE for 21 days. At least three of the congeners (BDEs-197, -201, and -207) were recovered in higher amounts than could be accounted for by their presence in the dose (>160%). The formation of lower brominated congeners from BDE-209 has been reported in fish. Carp (*Cyprinus carpio*) exposed to BDE-209 in the diet for 60 days accumulated penta- through octa-BDEs in their tissues but not BDE-209 (*11*). Rainbow trout fed dietary BDE-209 for more than 100 days accumulated

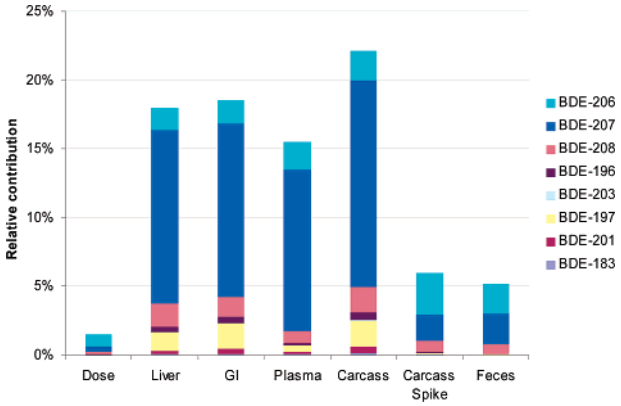

FIGURE 1. Relative contribution of individual hepta- to nona-BDE congeners to the total PBDE content of the DecaBDE formulation, the liver, GI tract, plasma, carcass, and feces of rats dosed for 21 days with DecaBDE, and a control rat carcass spiked with DecaBDE.

hepta- through deca-BDEs in their tissues (*7*, *12*). The presence of lower brominated congeners was attributed to metabolic debromination of BDE-209; the major octa and nona congeners were identified as BDE-201, -202, and -208 (*12*). Although reductive debromination of BDE-209 has not conclusively been observed in rats, trace amounts of nona-BDEs were found in rat tissues after a single oral dose of ^{14}C-labeled BDE-209 (*6*). It is possible that metabolic debromination can occur at a low rate in rats and that these congeners magnify and are retained to a larger extent than the BDE-209 due to differences in uptake and half-lives following 21 days of dosing. Another explanation for their presence is the degradation of BDE-209 during tissue extraction and cleanup because, under photolytic conditions, BDE-209 is known to degrade to lower (tetra to nona) brominated diphenyl ethers (*20*).

Debromination. Although precautions were taken to minimize exposure to light in this study, the possibility of photolytic or chemical debromination was investigated. To determine whether the excess BDEs-197, -201, and -207 in tissues were due to degradation of BDE-209 during cleanup, samples of the control carcass were spiked with the DecaBDE formulation at a concentration similar to what was found in the dosed rat carcasses on day 0. The fortified samples (n = 3) were processed by the same cleanup procedure and analyzed by the same GC/MS method as all other samples. Concentrations of BDEs-197, -201, and -207 in the spiked tissues were approximately 10-fold lower than in the carcasses of dosed rats (Table 1) and could not account for the excess amounts recovered. In addition, the pattern of nona- and octa-BDEs observed after the cleanup of spiked tissue did not resemble the pattern of congeners retained in the tissues of the dosed rats (Figure 1). These results demonstrated that the cleanup method did not cause significant degradation of BDE-209 to BDE-197, -201, or -207 (<2% of BDE-209 present in the tissue) and also showed no measurable amounts of octa-BDEs formed. In the dosed rats, BDEs-207 and -197 were preferentially formed.

Figure 1 also shows that while the hepta- to nonasubstituted congeners constituted only 1.5% of the dose, these congeners accounted for 16−22% of the total PBDEs found in the tissues, with BDE-209 making up the remaining portion. Distribution of the octa to deca congeners was proportionate throughout the rat as shown by the similar congener patterns in each tissue compartment. BDEs-207 and -197 were the major isomers in the nona and octa homologue groups, respectively. On the basis of the observations of greater than 160% recovery and higher than predicted relative proportions, BDEs-197, -201, and -207 may be forming from metabolic debromination of BDE-209, albeit to a small extent because the excess recovered mass represents an estimated 1% of the BDE-209 dosed. Concentrations of BDE-183 were quite low in all tissues, making it difficult to conclude whether this congener was forming from debromination.

The formations of BDEs-197 and -207 from BDE-209 result from *meta*-debromination(s), and formation of BDE-201 results from *para*- and *meta*-debrominations. In fish the major debromination products of BDE-209 were due to *para*-losses (i.e., BDEs-208 and -202) and *para*- and *meta*-losses (i.e., BDEs-201 and -188) (*12*). Anaerobic microbes in sewage sludge have also been shown to debrominate BDE-209 mainly via *para*- and *meta*-losses to octa- and nona-BDEs (*21*). The intestinal tract is a rich source of anaerobic microflora, so gut bacteria may be the site of BDE-209 debromination in rats and fish. However, fish liver microsomes also have the capability of debrominating BDE-209, pointing to hepatic enzymes such as deiodinases as the metabolic mechanism (*12*). Deiodinase enzymes generally catalyze the *meta*-dehalogenation of the thyroid hormone thyroxine (L-3,3′,5,5′-tetariodothyronine, T_4) to triiodothyronine (L-3,3′,5-triiodothyronine, T_3) or reverse triiodothyronine (L-3,3′,5′-triiodothyronine, rT_3) and are present in many tissues in the body. In this study the rather equivalent distribution of PBDEs among all tissues (liver, GI tract, plasma, and carcass) may suggest a more diffuse metabolic route for debromination such as the deiodinase enzymes rather than gut microflora, but further studies are needed to support this hypothesis.

Mass Balance. The amount of unchanged BDE-209 retained in the tissues and plasma of the rats after 21 days of dosing was roughly equivalent to one daily dose (5% of the total dose or 3.6 μg). In an attempt to account for the remainder of the dose, the amount of BDE-209 excreted in the urine and feces during the entire dosing period was measured. The urine contained no BDE-209 or other PBDEs at concentrations above the detection limits or, at most, 0.03% of the dose. The feces from dosed rats contained mainly BDE-209, and nona-BDEs comprised 5% of the total PBDEs (Table 2 and Figure 1). A comparison of the relative distribution of octa-, nona-, and deca-BDEs in the feces with that of the spiked carcass makes it unclear whether the octa and nona congeners were actually excreted in the feces or whether they were formed during the cleanup procedure (Figure 1). The amount of BDE-209 recovered in the feces was <4% of the total BDE-209 dose, leaving 90% of the BDE-209 unaccounted for (Table 2). Previous researchers have shown that 30% of a single ^{14}C-BDE-209 dose was not absorbed and excreted in feces as parent compound, while the remainder was converted to bound residues and hydroxylated metabolites by rats (*6*). The present experiment tends to suggest a similar fate for BDE-209, but identification of bound or hydroxylated metabolites was not undertaken at this time; instead the focus was on reductive debromination to other PBDEs. The lower excretion of unchanged BDE-209 in this study compared to the previous study (*6*) was most likely due to more efficient uptake from multiple, smaller doses, supporting observations that uptake of BDE-209 may be dose dependent (*5*).

BDEs-206 and -203 were also incompletely accounted for in tissues and excreta, suggesting extensive metabolism. Debromination to lower substituted congeners was not directly observed for these two PBDEs, but it cannot be ruled out as occurring to a small degree. Formation of bound residues or hydroxylated metabolites may also have occurred. Studies to further investigate the metabolism of these individual congeners are planned for the future.

Depuration. A final objective of this study was to determine the whole-body depletion kinetics of BDE-209 in rats. A previous study in rats showed BDE-209 was quickly depleted with a terminal half-life in plasma of 2.5 days (*10*). In gray seals (*Halichoerus grypus*) fed BDE-209 for 30 days,

TABLE 3. Half-Lives Calculated for Select PBDEs in Rats Dosed with a DecaBDE Formulation for 21 Days Followed by 21 Days of Withdrawal[a]

tissue	BDE congener	model[b]	half-life (days) α	b	95% CI	R^2
carcass	209	first-order	8.6	na	6.1−14.6	0.747
	208	first-order	14.9	na	10.0−29.8	0.565
	207	first-order	22.6	na	15.4−42.6	0.608
	206[c]	first-order	7.4	na	5.0−13.8	0.694
	203[c]	second-order	1.6	64.8		0.842
	201[c]	not modeled[d]				
	197	not modeled[d]				
	196[c]	second-order	1.0	62.6		0.913
liver	209	second-order	0.7	20.2		0.897
	208[e]	first-order	5.9	na	5.1−7.1	0.950
	207	first-order	4.7	na	3.3−8.0	0.777
	206[e]	second-order	1.2	16.3		0.535
	203	second-order	1.0	11.6		0.865
	201	first-order	6.1	na	4.4−9.7	0.783
	197	first-order	6.9		5.0−11.0	0.771
	196	first-order	4.4		3.3−6.8	0.831
plasma	209	second-order	1.2	75.9		0.886
	209	first-order	3.9	na	2.7−6.8	0.753
	207	first-order	7.9	na	5.4−14.7	0.708

[a] Data were fit to either a one-phase exponential decay curve to yield a single half-life or a biphasic decay curve to yield α and β half-lives. Correlation coefficients (R^2) for each curve and 95% confidence intervals (95% CIs) for single half-lives are given. na = not applicable. [b] Data were fitted to either first-order or second-order depletion curves. [c] Data for one of the rats at withdrawal day 14 was removed as an outlier (>2.7 times the average of the other two values at that time point). [d] Not modeled due to unsuitable fit with any withdrawal equation. [e] Data for one of the rats at withdrawal day 0 was removed as an outlier (>3.2 times the average of the other two values at that time point).

the apparent half-life in blood was estimated at 8−13 days using a first-order model; however, elevated levels of BDE-209 remained in the blubber after 29 days of withdrawal (*8*). The role of body mass and body fat is known to be important in the elimination of lipophilic compounds such as dioxins and PCBs (*22*). In an extreme case, PCB-153 (2,2′,4,4′,5,5′-hexachlorobiphenyl) has been shown to rapidly redistribute from the blood and liver of rats but to almost irreversibly deposit into the skin and adipose tissue (*23*). Therefore, it becomes important to determine whole-body half-lives of lipophilic compounds that may deposit or repartition into lipid stores.

Table 3 shows the calculated half-lives for BDE-209 in plasma, liver, and carcass (sans the GI tract). The plasma half-life was calculated on a concentration basis (pg/g of plasma) because total recovery of blood was not possible; the data were best fit to a biphasic depletion curve. The distribution phase (α) had a relatively short half-life (1.2 days), in agreement with previous studies; however, the elimination half-life (β = 75.9 days) calculated in this study was much longer than reported in a previous study using a three-compartment model (2.5 days) (*10*). This long elimination half-life may reflect slow elimination of residual body stores of BDE-209 following chronic dosing; however, it could also contain significant error due to the relatively short withdrawal time, the small number of animals, and animal-to-animal variations.

When the BDE-209 plasma data were fit to a one-compartment model, the BDE-209 half-life was 3.9 days (95% confidence interval 2.7−6.8 days), similar to half-lives previously calculated for rats and gray seals (*8*, *10*), but the correlation was not as good as with the second-order model. Only one other congener (BDE-207) had plasma levels elevated enough to produce a reliable withdrawal curve. Table 3 shows that the half-life of BDE-207 in plasma was twice that of BDE-209 when both were modeled as single compartments.

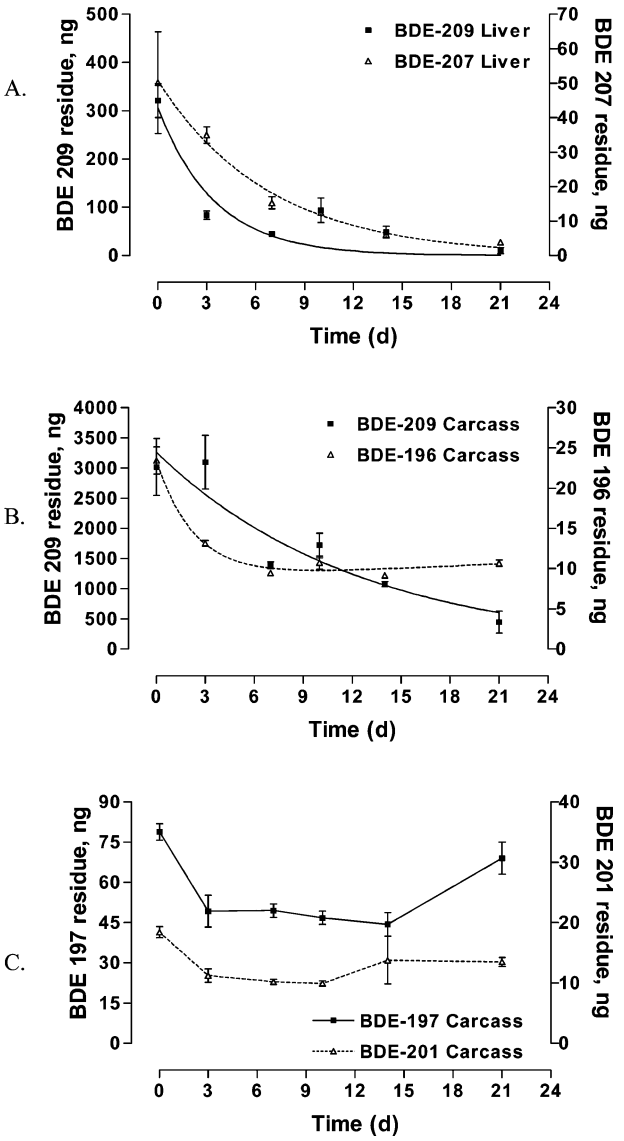

FIGURE 2. Depletion of various PBDEs from rats dosed with a DecaBDE formulation for 21 days and slaughtered in groups of three at withdrawal periods of 0, 3, 7, 10, 14, and 21 days. Lines show the modeled withdrawal curves for (A) total nanograms of BDE-209 (—) and BDE-207 (---) in the liver, (B) total nanograms of BDE-209 (—) and BDE-196 (---) in the carcass, and (C) total nanograms of BDE-197 (—) and BDE-201 (---) in the carcass. Symbols represent the average value at each time point with error bars showing the standard deviation; only two values were included for BDE-196 in the carcass at day 14.

Carcass and liver depletion kinetics were modeled for BDE-209 and the major nona-and octa-BDEs using total tissue residues (ng of PBDE in the tissue) to eliminate the effect that changes in body mass might have over the course of the withdrawal period (Table 3 and Figure 2). For BDEs-209, -206, and -203 depletions from the liver followed biphasic curves as exemplified in Figure 2A for BDE-209. The half-lives of the distribution phases (α) were approximately 1 day with elimination half-lives (β) of 10−20 days. For the PBDEs that may be forming due to debromination (BDEs-207, 201, and 197), liver depletion rates were constant (i.e., best modeled using one-compartment) as represented by BDE-207 in Figure 2A and may reflect continued formation of these congeners from residual BDE-209.

Residue depletion of the PBDEs from the carcass varied depending on the congener but could be characterized by three different scenarios: (1) single-phase depletion as

represented by BDE-209 in Figure 2B, (2) biphasic depletion as represented by BDE-196 in Figure 2B, or (3) U-shaped "depletion" as illustrated for BDEs-197 and -201 in Figure 2C. Whole-body depletion of BDE-209 was more than twice as long as from the plasma and had a half-life of 8.6 days. For the nona-BDEs, BDE-206 had a half-life on the order of that of BDE-209 (7.4 days) while BDE-208 and -207 half-lives were 2 and 3 times longer, respectively. A similar trend has been observed in exposed workers where serum half-lives ranged from 15 days for BDE-209 to 39 days for BDE-207 (*16*). The longer residence of BDEs-207 and -208 in the body may be due to continued formation from BDE-209 or greater resistance to metabolism than that of BDE-206.

For the octa-BDEs, BDEs-203 and -196 appeared to follow a biphasic depletion curve with a short distribution phase (α < 2 days) and a longer elimination phase (β > 60 days). This indicates that certain compartments in the body may have a higher affinity for these compounds, leading to a slower release over time. Two of the octa-BDEs (201 and 197) could not be modeled for depletion because amounts actually appeared to increase in the carcasses after 3 weeks of withdrawal (Figure 2C). This supports the hypothesis of formation via debromination of BDE-209. Future studies with individual congeners would give more accurate estimates of half-lives.

In general, the half-lives of the PBDEs observed in this study were shorter in the liver than the carcass, reflecting the higher perfusion and/or metabolizing capability of this organ. Half-lives increased as the degree of bromination decreased from deca- to octa-BDEs. Some of the whole-body elimination rates were on the order of half-lives calculated for tetra- to hexa-BDEs in the adipose tissue of male rats (19−120 days) (*24*) or the elimination half-lives of other persistent lipophilic compounds in rats such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (18.7 days) and 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (30.9 days) (*22*). While the withdrawal time chosen for this study was sufficient for BDEs-209 and -206 whole-body half-life determinations (over twice the half-life), it was too short for accurate calculations for some of the congeners, and these should be considered estimates. By the end of the withdrawal period octa- and nona-BDEs made up 54% of the PBDE body burden in the rats as compared to 22% at the end of the dosing period and 1.5% of the original dose, demonstrating not only the increased persistence of certain octa- and nona-BDEs but also their putative metabolic formation from BDE-209. Unlike many persistent pollutants, metabolism of BDEs-209, -206, and -203 appeared high as shown by incomplete mass accountability and low bioconcentration into the carcass. Because the methods used in this study selectively analyzed for PBDEs, no identification of other potential metabolites such as hydroxyl compounds or bound residues was made, and these may account for a majority of the dose. Future studies are planned to focus on this area.

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Supporting Information Available

Further details of the experimental methods and congener identification. This material is available free of charge via the Internet at http://pubs.acs.org.

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